Individual variation in lateral septal vasopressin

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General Discussion

The experiments performed in this thesis were aimed at the relationship between lateral septal vasopressin (VP) and coping style. First we focussed at the question whether the *correlation* between lateral septal VP and coping style exists in the rat, in particular the wild-type rat, thereby suggesting that it may be a phenomenon present in more species. Secondly, a number of experiments were aimed at the *causal* relationship between lateral septal VP and aspects of coping behaviour.

1 SUMMARY OF RESULTS

In **Chapter 2**, the differential VP distribution was studied in males of an outbred strain of wild-type rats in relation to their level of intermale aggression. Biochemical and immunocytochemical data revealed a negative correlation between lateral septal VP and aggression. Aggressive rats exhibited low levels of VP whereas intermediate and nonaggressive animals showed a higher amount of the peptide together with a more widespread distribution of VP-immunoreactive (VP-ir) fibres. No differences in adult levels of plasma testosterone (T) were found. The results confirm the hypothesis that the variability in lateral septal VP, originally described as a sexual dimorphism, can be found in male rats as well. Behavioural differentiation in wild-type rats was studied in **Chapter 3**. The rats were selected on basis of their intermale aggression and tested in the shock-probe bury test, the Morris water maze, and the 8-arm radial maze. The results of the first test showed that aggressive males respond actively (i.e. bury the electrified probe), whereas nonaggressive rats show withdrawal and hardly any burying. The results of the spatial tasks in general failed to show a clear relation between aggression and spatial performance. It is concluded that the existence of the behavioural discernable coping styles is evident in the shock-probe bury test, but does not prevail in these spatial tasks. Chronic septal infusion of a V_2/V_1 receptor antagonist was used to test the role of VP in social memory, anxiety-related behaviour, and spatial memory (**Chapter 4**). Blocking the action of lateral septal VP resulted in impaired social memory and increased anxiety on the elevated plus-maze. No behavioural effect of the treatment was found in shock-probe burying and spatial learning in the Morris water maze. Overall, these results suggest a strong task-depending specificity of lateral septal VP modulation. In **Chapter 5** we investigated whether the role of VP in social recognition is specific for this social setting or that

it may have an effect on general investigatory behaviour as well. In a paradigm identical to social recognition the rats were tested on their ability to recognize objects instead of juveniles. The data showed that, in contrast to social recognition, object recognition reflects a form of habituation. In addition, object recognition was not influenced by VP receptor antagonist treatment.

2 VARIABILITY IN LATERAL SEPTAL VASOPRESSIN

Both biochemical data and immunocytochemical data revealed a considerable variation in VP in the lateral septum (LS) of wild-type rats (Chapter 2). This variability within the male gender was also found in mice by Compaan et al. (1993). It seems that the most extreme cases of the VP-ir found in the wild-type rat (see Chapter 2, Fig. 4) resemble average distribution in the aggressive and non-aggressive selection lines. However, it is difficult to make a more detailed comparison of the variability in VP between the present study in rats and the study in mice, because different parameters were used to illustrate VP expression in both studies. The presence of the same variability in male rats as found in mice strongly suggests that it is a more general phenomenon, which might be detected in more species. Moreover, by studying the differentiation in a strain of outbred rats, the assumption was confirmed that an unselected population exhibits the variability in VP and that it is not a by-product of genetic selection.

The septal VP fibres originate from neurons in the bed nucleus of the stria terminalis (BNST) and the medial amygdala (MeA). One might expect therefore that a differentiation in the number of VP-ir cells in the (BNST) might exist in the rats. This has been studied in the mice and individuals from the nonaggressive line were found to express more VP-ir cells than animals from the aggressive line (Compaan et al. 1993). The MeA, which is believed to form a vasopressinergic unity with the BNST (Caffé and Van Leeuwen, 1983; Caffé et al. 1987) might express the differentiation as well. This has not been determined in the selection lines of mice. Normally, the low level of VP in the cells of the BNST and the MeA necessitates colchicine treatment (axonal transport blockade) in order to immunostain VP-expressing cells. However, the treatment was incorrect in the present study and it resulted in unreliable counts of the number of VP-positive BNST cells. Since the MeA is located even further away from the colchicine-injection site than the BNST, the accessibility of these cells to colchicine was insufficient and dit not result in any staining in the amygdalar area. Studies on sexual dimorphism in both areas consistently showed males to have more VP expressing cells than females (Miller et al. 1989b; Wang et al. 1993; De Vries et

al. 1994; Szot and Dorsa, 1994; Wang and De Vries, 1995). When combining these data with the findings on differential cell number in the BNST within the male gender of mice, one might suggest a difference in the number of VP-expressing cells in the MeA as well. To reliably confirm the hypothesis that the differential VP distribution in the male gender is a general phenomenon, this is one issue that still has to be studied.

Although the observed variability in VP has now been demonstrated in both rats and mice, one cannot simply extrapolate this to every species. As reviewed in Chapter 1, lateral septal VP expression has many "faces" depending on the species studied. For instance, there is a substantial variation in basal lateral septal VP-ir when males of several vole species are compared (Wang et al. 1996). In the LS of males from the pine vole about a fifth of the total sample area was covered by VP fibres, whereas these fibres exhibit no more than a fifteenth part of the area in males of the meadow vole. The fibre distribution of the latter resembles the low VP distribution observed in the most aggressive rats examined in this thesis. When the average fibre density in males of a certain species is already low, the possible variability within these males will be small as well and therefore difficult to discern. Apart from individual differentiation in distribution, the lack of almost any VP-ir neuron in the BNST or fibre in the LS of Syrian and golden hamsters indicates that also large species differences in the male may exist (Albers et al. 1991; Delville et al. 1994). Next to the question whether the male variability is present in more species it would be equally interesting to study which processes induce the variation in VP expression and whether these processes have a general character. They may be the same mechanisms which underlie sexual dimorphism.

It is remarkable that the most extreme examples of VP distribution in the aggressive rat seem to resemble normal female distribution, whereas the abundance of fibres in a nonaggressive rat seems even larger than normal male distribution (De Vries et al. 1981; Wang et al. 1993). The data of the selection lines of aggressive and nonaggressive mice (Compaan et al. 1993) show the same tendency when VP-ir cell numbers in the BNST are compared (Miller et al. 1989b; Wang et al. 1993; De Vries et al. 1994). On face value, photomicrographs presented in a study by De Vries et al. (1981), which show a survey of the LS of an adult male and female rat, closely resemble the photomicrographs shown in Chapter 2 of this thesis (Fig. 4). However, it should be noted that comparing the variation among males with variation between the sexes is, again, difficult because most studies use different staining protocols, primary antibodies, and parameters to illustrate their results.

Most of the literature describing VP distribution did not consider the variation of VP expression in the male gender reported in the present thesis. This

may be due to the use of inbred lines of rats in the majority of these studies. Most frequently used animals are the Wistar rat and the Long-Evans rat (De Vries et al. 1985; Van Leeuwen et al. 1985; Caffé et al. 1987; Miller et al. 1989b; Miller et al. 1992; Wang et al. 1993; De Vries et al. 1994). The use of these laboratory lines may have obscured the naturally occurring variation as observed in wild-type rats.

There are few other reports which have studied differences in VP content in brain areas of untreated male animals. Ermisch et al. (1986) found VP levels to be significantly higher in the septum/striatum and posterior pituitary of Wistar rats with high performance in a brightness discrimination (BD) task compared to the group of low performers. However, in a later study using Wistar rats which were genetically selected for their BD-performance this could not be replicated. Instead, a difference in hippocampal VP content was reported (Hess et al. 1992). These data show the involvement of a genetic factor in VP expression in the hippocampus, but the selection criterion seems to be only loosely coupled (or not at all) to septal VP. The Roman high- and low-avoidance rats (selected and bred for rapid versus non-acquisition in two-way, active avoidance behaviour) have also been found to express differential basal levels of VP (Aubry et al. 1995). However, these differences were found in the parvicellular neurons of the paraventricular nucleus, i.e. the low-avoidance group showed high levels of VP. House mice selected for differences in nest-building behaviour have been reported to differ in the number of VP-ir neurons in the suprachiasmatic nucleus (SCN) (Bult et al. 1992; Bult et al. 1993). Low-nesting animals have a higher number VP-expressing cells than high-nesting and control mice. Sluyter and co-workers (1995) have shown that selection for nest-building convincingly correlates with selection for aggression. Subsequently, it was shown that males from the nonaggressive selection lines have higher number of VP-ir cells in the SCN than aggressive animals (Compaan, 1994, personal communication). Altogether, these data indicate that, within the male gender, differentiation in VP neurons might be more widespread throughout the brain. Under the notion that the variability in VP in an unselected population is not a by-product of genetic selection in the laboratory, the more widespread differentiation may thus be an issue of investigation in wild-type rats as well.

One may wonder what this means for the total VP signalling in the lateral septal area. The observed differences may be due to a differential rate of release or storage of the peptide in the LS. It remains to be seen whether high peptide content as measured via RIA also means higher rates of peptide release. In the present study it was shown that in the nonaggressive individual the fibre network is more widespread. It may thus be that the higher *fibre density* per septum is responsible for the higher levels of VP measured by RIA. The data might

suggest that per VP-ir fibre there is at least an equal amount of peptide present. However, optical density measurements obtained from mice (Compaan et al. 1993) showed that more VP is present in fibres of nonaggressive animals compared to aggressive males. The use of colchicine in the present study does not allow measuring optical density, but we may reasonably assume the nonaggressive wild-type rat to have more peptide per fibre than aggressive individuals.

The studies on the role of T on VP expression indicate that the VP-ir in the lateral septal fibres reflects storage levels. When adult male rats are gonadectomized, a significant decrease in the VP fibre density in the LS occurs, which takes several weeks before it is completely disappeared. This is preceded by a significant decrease in the expression of cytoplasmic and nuclear VP mRNA in the BNST (and MeA) within 3 days (De Vries et al. 1985; Miller et al. 1992; Szot and Dorsa, 1994). All these effects can be restored by replacement of T; it takes 3 days of T replacement for the number of cells expressing VP mRNA to return to control level, whereas the amount of labeling per cell requires 7 days of T treatment. Five weeks of T replacement was shown to restore VP-ir and content to control levels in both the BNST and the LS (De Vries et al. 1984; De Vries et al. 1985; Van Leeuwen et al. 1985; Miller et al. 1989a; Miller et al. 1989b; Szot and Dorsa, 1994; Magnusson and Meyerson, 1996). These studies point to low basal release of VP indicating that the newly synthesized peptide may remain stored within the cell and its processes for prolonged periods of time. Estimates from neural lobe disappearance of VP indicate a half-life of approximately 19 days, but it is unknown whether VP in the extrahypothalamic areas degrades or disappeares with a similar time course (Burford and Pickering, 1973). Taken together, aggressive wild-type rats exhibit relatively low levels of lateral septal VP-ir, i.e. storage. This may also suggest that these animals show higher levels of release when compared with their nonaggressive counterparts. This is subscribed to by a study of Landgraf and co-workers (1988), which shows that low contents of VP in distinct brain areas may well be linked to stimulated release patterns. Opposed to this, one would expect that VP released from a widespread and abundant fiber network (nonaggressive animals) would result in a larger effect than VP released from only a limited number of fibers (aggressive animals). Hence, on basis of these arguments it seems impossible to draw a conclusion on the existence of a differential VP signalling in the lateral septal area. Future VP mRNA studies could supply additional knowledge, but one would still lack information on translation rates to produce the peptide from its mRNA. Differences in lateral septal V_{1a} receptor subtype sensitivity and distribution are not expected to occur within male wild-type rats. It is known that VP binding sites are not sexually dimorphic and gonadectomy or T treatment at adult age does not effect the density or affinity of

the VP binding sites (Tribollet et al. 1990; Poulin and Pittman, 1991). However, recent observations show that the Roman high- and low-avoidance selection lines exhibit a differentiation in V_{1a} receptor distribution in the central amygdala and paraventricular nucleus (PVN; A. Johnson et al. 1997, personal communication). This indicates that, next to differences in syntheses, storage, and release presynaptically, we may be confronted with a differentiation in postsynaptic mechanisms as well. At this stage, a discussion on this subject would be to speculative and is considered out of focus in the present thesis.

The final issue in this paragraph concerns the origin or induction of the differential lateral septal VP distribution. At adult age, VP expression is dependent on the presence of T or its metabolites (see General Introduction, paragraph 2.3.2). The lack of a significant correlation between adult levels of plasma T and VP-ir as shown in Chapter 2 indicates that adult T does not explain the differential distribution in this instance. It thus seems reasonable to assume that the organizational processes occurring pre- and postnatally may play a role in inducing the differentiation. As described in the General Introduction, T exerts an organizational role in sexual dimorphism in VP distribution at neonatal age, rather than at adult age (Wang et al. 1993; Wang, 1994). Neonatal T seems to be an important factor in the males of the selection lines of wild house mice as well. A single injection of T at postnatal day 1 reduced the VP-ir in adult nonaggressive males until levels similar to control aggressive mice (Compaan et al. 1997), submitted for publication). Other studies suggested an endogenous difference between aggressive and nonaggressive males in neonatal T sensitivity and peak levels (Compaan et al. 1992; De Ruiter et al. 1992; Compaan et al. 1994b). This closely resembles mechanisms which are thought to underlie sexual differentiation in the same VP network (De Vries and Al-Shamma, 1990; Szot and Dorsa, 1993; De Vries et al. 1994; Szot and Dorsa, 1994). Thus, whereas the VP differentiation within the adult male gender seems to be as large as the sexual differentiation in VP in the same brain areas, the mechanisms causing both types of differentiation neonatally seem to be identical as well.

It remains to be established whether these neonatal processes are the only cause of the observed variation in VP. It may be that environmental circumstances around birth or maybe even in later life influence basal VP expression in the wild-type rats. Recently, it was shown that chronic or short inescapable stress produces long-lasting changes in VP expression in hypothalamic areas (zona externa of the median eminence) in adult male rats (De Goeij et al. 1992a; De Goeij et al. 1992b; De Goeij et al. 1992c; Van Dijken et al. 1993). In fact, corticotropin-relasing factor (CRF)-expressing cells start co-expressing VP, resulting in an increase in VP content whithout alteration of CRF stores. Moreover,

there is an increased release of VP relative to that of CRF as a response to these forms of stress. Whether these stress-induced processes play a role neonatally and in the MeA-BNST-LS system as well is unknown. It was suggested that galanin, instead of CRF, might regulate the coexpression of VP in these areas (see General Introduction, paragraph 2.4.1, (Planas et al. 1994b; Al-Shamma and De Vries, 1995; Planas et al. 1995). Perhaps the pups which will devellop as nonaggressive individuals are more susceptible to neonatal stress and that similar processes influence basal VP expression. Although very speculative, this might partially account for the heterogenity in the radioimmunoassay data of the nonaggressive group (Chapter 2).

3 LATERAL SEPTAL VASOPRESSIN AND AGGRESSION

The differential lateral septal VP-ir and content correlated with the level of intermale aggression measured in a standard resident-intruder test. Aggressive males were shown to have low levels of VP whereas intermediate and nonaggressive animals showed higher levels. This paragraph will discuss whether this is a causal relationship (i.e. high levels of VP reduce aggression) or that we found just a correlation between VP and aggression without any causality.

The role of lateral septal VP in aggressive behaviour has been studied in a number of species. For instance, several studies showed that VP, when applied to the LS, is able to increase aggression-related flank marking in golden hamsters (Ferris et al. 1993; Ferris et al. 1994; Ferris et al. 1996). However, this result seems difficult to interpret since the LS of golden hamsters is almost devoid of VP immunoreactive fibers (Ferris and Delville, 1994). The presence of the V_1 receptor in the LS (Ferris et al. 1993) of golden hamsters indicates the likeliness of a functional system. Another example in which lateral septal VP is implicated in aggression is found in microtine rodents. In the monogamous prairie vole, lateral septal VP is strongly related to intermale aggression (Winslow et al. 1993; Wang et al. 1994b; Wang, 1995). In this species, VP antagonism seems to block the transition to aggression after mating. Furthermore, intracerebroventricular VP administration by osmotic minipumps in the LS, causing a threefold increase in VP locally, is able to induce intermale aggression within twelve hours. This effect of VP is strongly related to the process of pair bonding since in meadow voles, a species devoid of parental care, no changes in lateral septal VP were found after cohabitation with females or after becoming fathers (Bamshad et al. 1993; Wang et al. 1994a). Also, prairie voles exhibit both high levels of basal lateral septal VP-ir and aggression compared to meadow voles (Wang, 1995). Administration of VP

into the septum of rats has also been shown to enhance aggression (Koolhaas et al. 1991).

Thus, in most of these studies application of exogenous VP in the septum induces an increase in aggression. These data seem in contrast with the present results since we reported that high levels of VP correspond with low intermale aggression levels. However, as mentioned before, high VP-ir and peptide content may also be interpreted in terms of increased storage and low release of VP in nonaggressive animals. In this way, the results seem to be inconsistent with the assumed role of lateral septal VP in aggression, but at this moment there are no clear data to support this hypothesis. Until then, we are compelled to conclude that the differential distribution of basal VP in wild-type rats has no direct causal link to intermale aggression.

It should be kept in mind that aggression exists in many forms and may serve different functions, depending on species and situation. For instance, the mating-related form of aggression in voles is transient of nature, whereas the form of aggression a wild-type rat shows can be observed at any time. Hence, different neuronal systems may participate in concert with VP in the regulation of this behaviour.

4 LATERAL SEPTAL VASOPRESSIN AND COPING STYLES

Next to the relationship between lateral septal VP and aggression we investigated whether the observed differences in VP are reflected in other functional behaviours. Since aggression has been shown to be just one aspect of coping styles, we studied the relationship between differential VP expression and behaviours relevant to these styles. The concept of coping styles in relation to aggression has been recognized and studied in the selection lines of house mice (Van Oortmerssen and Bakker, 1981; Bohus et al. 1987; Benus et al. 1991b). There are indications that this characterization based on one trait (i.e. aggression) is applicable to the wild-type rat as well (Sgoifo et al. 1996). Therefore, described in Chapter 3, experiments were performed to expand the knowledge on behavioural differentiation in wild-type rats. In addition, we studied the role of lateral septal VP in behaviours in which wild-type rats were expected to differ (Chapter 4 and 5). Whether these results confirm a role for lateral septal VP in coping styles will be discussed here.

The differentiation in shock-probe bury (SPB) behaviour are in accordance with observations of Sgoifo et al. (1996) in wild-type rats and Sluyter et al. (1996) in wild house mice. In fact, these data have been expanded including

wild-type rats which show intermediate scores of aggression (Koolhaas et al. 1997b). The results consistently confirm the positive correlation between the level of aggression and the amount of burying behaviour. The aggressive animals respond actively to the situation and thus seem to adhere to an active coping style. The nonaggressive rats adopt a passive coping style towards the aversive stimulus in that they react with withdrawal. However, blocking the endogenous action of lateral septal VP by chronic application of a VP receptor antagonist did not have an effect on SPB behaviour (Chapter 4). We may thus conclude that lateral septal VP does not play a causal role in this coping style related behaviour.

Somewhat surprising, the results of the spatial tasks, in general, failed to show a clear relation between aggression and spatial performance in the wild-type rats (Chapter 3). In itself this suggests, although VP may still be involved, that a differentiation in lateral septal VP is not reflected in a differentiation in spatial learning abilities. Whether VP does play a role in spatial learning is under debate. Engelmann and co-workers showed that (exogenous) VP impaires spatial learning. However, the application of a VP antagonist has no effect on the performance of the animals (Engelmann et al. 1992) and Chapter 4).

Performance on the elevated plus-maze and the social recognition paradigm were shown to be influenced by local VP antagonism. They may thus be candidates to relate differential levels of VP with coping styles. Wild-type rats have been tested on the elevated plus-maze, but there does not seem to be a relation between their performance and coping styles. The social recognition paradigm, in its present form, appeared to be unsuitable for wild type rats; too many juveniles were killed by the adult residents during testing. The test was originally developed to study social memory in laboratory rats (Thor and Holloway, 1982). Killing unrelated juveniles seems to be a trait of wild rodents. Perhaps the process of domestication has changed most laboratory strains in such a way that they will exept unrelated juveniles in their neighbourhood. The object recognition paradigm described in Chapter 5 is not a good alternative, because it was shown that this behaviour is not under the control of lateral septal VP and cannot be considered as a suitable test to relate differential levels of VP with coping styles.

In summary, we first argued that a direct, causal link between the differential distribution of lateral septal VP and the level of intermale aggression is unlikely. Now, considering the main results of Chapter 3, 4, and 5, there is little reason to assume that the differentiation in VP is firmly related to a differentiation in coping styles. Furthermore, the question what the behavioural (and physiological) consequences are of a differential vasopressinergic innervation in the LS remains largely unanswered.

5 GENERAL REMARKS

Despite the fact that we could not find a direct causal link between lateral septal VP and coping behaviour, it remains remarkable that also other vasopressinergic systems seem to differentiate between coping styles. Two indications that VP does play a role in this differentiation of coping styles stems from work on the Roman rat lines. Aubry and co-workers have shown that basal VP mRNA levels in the parvicellular neurons of the paraventricular nucleus (PVN) were significantly higher in the Rla than the Rha rats (Aubry et al. 1995). VP injected into the central amygdala (CeA) effects cardiac and behavioural responses to stress in the Rla whereas the peptide is ineffective in the Rha rat (Roozendaal et al. 1992; Bohus et al. 1996). In addition, house mice selected for coping style related differences in nest-building behaviour have been reported to differ in the number of VP-ir neurons in the suprachiasmatic nucleus (SCN) (Bult et al. 1992; Bult et al. 1993). The function of these structures and the role of differential VP levels in these areas is perhaps better understood than VP's role in the LS. Apart from its function in social memory, its role in spatial memory and anxiety-related behaviour is not without debate. Clear hypothesis on the function of the LS is a prerequisite of studying VP functioning and the consequences of differential peptide levels.

The lack of clear results on the function of lateral septal VP may be due to the fact that we did not use VP itself in our studies. There is a disadvantage in the chronic application of VP, because local application may result in severe motor disturbances and epileptic fits. It is known that VP exhibits a self-sensitization phenomenon after repeated VP administration. Initial minor motor actions after a first application of relatively high doses are followed by enhanced motor disturbances including barrel rotations, myotonic convulsions and sometimes death (Poulin and Pittman, 1993a; Poulin and Pittman, 1993b). These effects are mediated through the V_1 receptor. A second unique feature of the LS is the positive feedback action of VP on its own release (Ramirez et al. 1990; Landgraf et al. 1991). Although not detected in the LS, the results suggest that this positive feedback is mediated through the V_2 receptor. The exact physiological significance of this phenomenon remains to be shown. To prevent the above-mentioned behavioural disturbances to occur we decided to initially study VP functioning using a receptor antagonist. This has the additional advantage that a dosage which fully blocks the receptor can be easily found.

Local infusion of agonists or antagonists into discrete brain areas ensures a selective administration and allows in principle the quantification of biologically active concentrations. The use of osmotic minipumps reduces

normally occuring stressfull handling during behavioural testing. However, despite the use of refined infusion techniques which utulize minipump-connected brain cannulas, push-pull cannulas, and microdialysis, it still remains difficult to address appropriate dosage questions. Conclusions using the agonist in particular should be based on dosage-response relationships. To prevent this question of dosage of agonist, the triggering of endogenous peptide release by an experimental stimulation of a connected brain area (e.g. by osmotic stimulation) may be a usefull tool (for review, see Landgraf, 1995; Engelmann et al. 1996).

The research on the role of lateral septal VP is further complicated because of its function as a neuromodulator: rather than an on/off switch, it acts in a more/less mode. In addition, it will probably act in concert with other peptides and hormones to establish an effect. As a final remark in this paragraph, it may thus be rewarding to briefly focus on the neuroanatomical aspects of the septum as a functional entity to further understand the results obtained in this thesis.

Most neuroanatomical aspects of the septum, of which the LS and the medial septum (MS) are the major components, have been excelllently reviewed by Jakab and Leranth (1995). The area is considered as an interface between telencephalic regions such as the hippocampus and the amygdala complex on the one hand, and hypothalamic and brainstem regions on the other hand (see Figure 1). The VP fibers from cells in the BNST and MeA terminate to a large extent on so called 'somatospiny neurons' in the LS, where they converge with the massive glutamatergic hippocamposeptal input (Jakab and Leranth, 1990b; Jakab et al. 1991; Jakab and Leranth, 1995). This important neuroanatomical finding could relate VP to spatial learning, since VP was shown to maintain long-term potentiation (LTP) and to increase glutamate-mediated responses in lateral septal brain slices (Joëls and Urban, 1985; Van den Hooff et al. 1989; Van den Hooff and Urban, 1990). Together, these data suggest the excitatory action of VP to take place on the somatospiny neurons, most probably through the V_{1a} receptors. However, blockade of this receptor did not interfere with spatial abilities (Chapter 4, Engelmann et al. 1992). Although spatial learning itself has been shown to induce LTP-like changes in the LS (Garcia et al. 1993), a role for VP in vivo remains unclear. An explanation might lie in the intrinsic connectivity of the septum. The integrity of the connection between the MS and the hippocampus is essential for spatial memory (Wenk et al. 1992; Poucet and Buhot, 1994) (review (O'Keefe and Nadel, 1987), but the reciprocal septohippocampal loop is not closed by the long-assumed massive LS-MS path. Recent studies found this latter projection to be extremely sparse (Leranth et al. 1992; Witter et al. 1992). This may be the reason that vasopressinergic influence on spatial learning is almost negligible.

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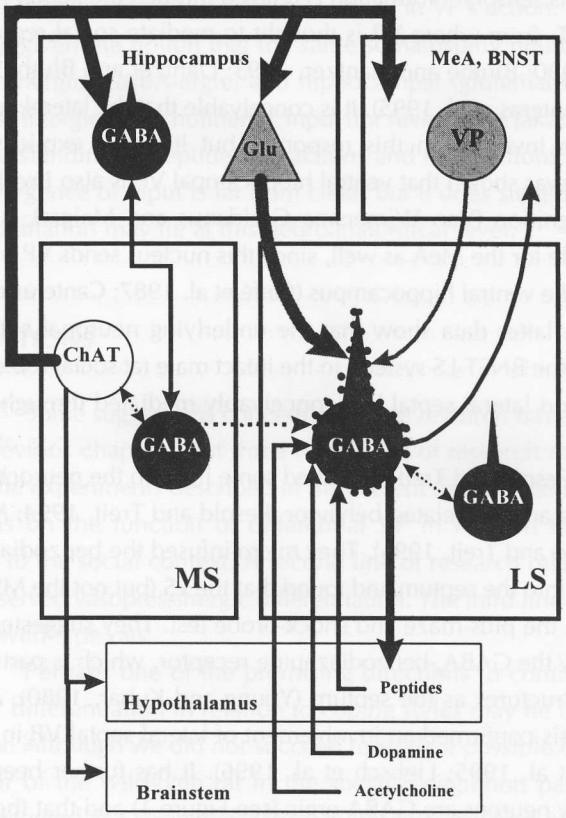

Figure 1: A simplified diagram outlines the bidirectional connections of neurochemically identified neurons and somatospiny neurons (cell with dots) in the medial and lateral septal divisions with other brain regions, in particular, telencephalic, hypothalamic, and brain stem areas. Dashed lines represent putative connections (based on indirect evidence). For further explanation, see text. BNST, bed nucleus of the stria terminalis; ChAT, choline acetyltransferase; GABA, γ-aminobutyric acid; Glu, glutamate; LS, lateral septum; MeA, medial amygdala; MS, medial septum; VP, vasopressin [Adapted from Jakab and Leranth, 1995].

With regard to the social recognition paradigm, some neuronal and anatomical data are known. Olfactory cues are important in social recognition and are sensed through the vomeronasal organ. In the adult male rat, this organ processes non-volatile chemoceptive stimuli from the ano-genital area of the

juvenile. This sensory information is relayed through the accessory olfactory bulbs to the BNST, from where VP is thought to mediate social recognition in the LS (Simerly, 1990; Bluthé and Dantzer, 1993; Dantzer and Bluthé, 1993; Gheusi et al. 1994; Canteras et al. 1995). It is conceivable that the lateral septal somatospiny neurons are involved in this response, but it is not explicitly demonstrated. Recently, it was shown that ventral hippocampal VP is also involved in regulating social recognition (Van Wimersma Greidanus and Maigret, 1996). This might suggest a role for the MeA as well, since this nucleus sends VP projections to both the LS and the ventral hippocampus (Caffé et al. 1987; Canteras et al. 1995). Thus, while these latter data show that the underlying neuronal substrate is not just confined to the BNST-LS system, in the intact male rat social recognition is strongly dependent on lateral septal VP, conceivably mediated through the somatospiny neurons.

Pesold and Treit have shed some light on the neurochemistry of the LS in regulating anxiety-related behavior (Pesold and Treit, 1994; Menard and Treit, 1996; Pesold and Treit, 1996). They micro-infused the benzodiazepine anxiolytic midazolam into the septum and found that the LS (but not the MS) suppressed fear reactions in the plus-maze and shock-probe test. They suggested this effect to be mediated by the $GABA_a$-benzodiazepine receptor, which is particularly abundant in limbic structures as the septum (Young and Kuhar, 1980). As discussed, the present thesis confirmed an involvement of lateral septal VP in anxiety behavior (Landgraf et al. 1995; Liebsch et al. 1996). It has further been shown that the somatospiny neurons are GABA-ergic (see Figure 1) and that they receive GABA-ergic, and, as mentioned before, vasopressinergic inputs (Jakab and Leranth, 1990a; Jakab et al. 1991; Jakab and Leranth, 1995). It may thus be assumed that the somatospiny neurons contain the $GABA_a$-benzodiazepine receptors implicated in anxiety-related behavior. Moreover, these cells may be the substrate where benzodiazepines and VP act to regulate anxiety responses, at least in the plus-maze. Shock-probe burying was not effected by VP antagonism, but can be influenced by benzodiazepines in the septum (Pesold and Treit, 1994; Pesold and Treit, 1996). Since the GABA-ergic somatospiny neurons are only a part of the lateral septal GABA-ergic cell population, one might suggest that the behaviour observed in the latter test is mediated in part by non-somatospiny neurons. However, it remains extremely difficult to speculate on the mechanism of the septum in these tests, mainly because of the abundance of GABA-ergic lateral and medial septal neurons and the existence of complex intraseptal connections which suggest a GABA-ergic crosstalk (Jakab and Leranth, 1990a).

Thus, whereas all these anatomical studies indicate the somatospiny neurons to be part of a collective, neuronal substrate involved in a variety of tasks,

our present results showed a strong specificity in VP's action. This may not be that surprising given the notion that the same somatospiny neurons receive not only vasopressinergic, GABA-ergic, and hippocampal (glutamatergic) input, but also catecholaminergic, and cholinergic input (for review, see Jakab and Leranth, 1995). Our understanding of peptide interactions and modulations which may occur at this convergence of input is far from clear, but it does suggest that the specificity of VP modulation may lie at this neuroanatomical level.

6 FUTURE DIRECTIONS

Some suggestions concerning future research have already been made in the previous chapters, but three main lines of research may be continued on basis of the experiments described in the present thesis. One line of research may still focus on the function of differential VP in relation to coping styles, but confined to the social context. A second line of research relates to the generality of the observed vasopressinergic differentiation. The third line concerns the coping styles in wild-type rats.

Perhaps one of the promising directions to continue studying lateral septal VP differentiation in relation to coping styles may lie in VP's role in social behaviour. Although we did not succeed to study a possible differentiation in the behaviour of the wild-type rat in the social recognition paradigm, recognition could be studied in a social defeat test. In this test, in which individual recognition is involved, animals are placed in the home cage of a dominant male conspecific. The introduction into the home cage of the resident will result in an aggressive interaction and a social defeat of the intruder. It has been shown that wild-type rats differentiate in their physiological responses to such an interaction (Sgoifo et al. 1996). Whether VP plays a role in this behaviour might be studied in a paradigm comparable to social recognition. Blocking lateral septal VP functioning before re-exposure to the same dominant male may result in a faillure to recognize this male. This may cause the intuder to show a behavioural pattern of an animal naive to the intruder and the situation. If VP is not involved, the intruder will immediately respond with submissive behaviour towards the resident male.

A further investigation into the role of VP in social behaviours can be derived from studies on the social organization in voles (or microtine rodents). In these species, VP has been implicated in pair bonding, maternal and paternal care, and the level of intermale aggression (Winslow et al. 1993; Bamshad et al. 1994; Wang et al. 1994a; Carter et al. 1995; Wang et al. 1996). It may thus be rewarding to further investigate social behaviours in relation to VP in the rat.

As mentioned, a second line of research relates to the generality of the observed vasopressinergic differentiation. In paragraph 3 of this chapter we concluded that the presence of the variability in VP expression is a relatively general phenomenon, which might be detected in more species. Also, it was suggested that it would be interesting to study which processes induce the variation in VP expression and whether these processes have a general character which may resemble mechanisms underlying sexual dimorphism. These mechanisms have always been presented as a special or unique feature of sexual dimorphism. However, one might suggest that sexual dimorphism is only a special case of brain differentiation in general. A variety of peptides, including galanin (Bloch et al. 1992; Planas et al. 1995), substance P (Malsburry and McKay, 1987; Malsburry and McKay, 1989), and dopamine (Beyer et al. 1991) are known to have a sexual dimorphic distribution. Several studies have shown that males which differ in their style of coping also exhibit a correlated differentiation in the central nervous system (Driscoll et al. 1990; Cools et al. 1993; Bohus et al. 1996; Koolhaas et al. 1997a; Koolhaas et al. 1997b). In particular, dopamine sensitivity has been found to differ between animals of both coping styles (Benus et al. 1991a; Rots et al. 1996). Detailed studies are necessary to investigate whether identical processes innitiate the differentiation between both genders and within one (male) gender.

Some steps to study this possibility have already been taken. Both the differentiation within the male gender and the sexual differentiation are mainly established pre- and postnatally. We have studied the effect of postnatal manipulation (injection of T, VP, corticosterone) on lateral septal VP content at adult age in Wistar rats, but the results are inconclusive (unpublished results). Nevertheless, Compaan and co-workers have shown that, in the wild house mouse, a single injection of T at postnatal day 1 is able to reduce the VP-ir in adult nonaggressive males until levels similar to control aggressive mice (Compaan et al. 1997), submitted for publication. One obvious aim of further studies on the generality and inducing mechanisms of VP distribution might be to include female individuals as well.

Finally, a third line of investigation concerns the presence of coping styles in wild-type rats. Although some studies have shown a behavioural and physiological differentiation (Sgoifo et al. 1996; Koolhaas et al. 1997b), apart from the presently observed differentiation in lateral septal VP, evidence of other neuronal differences are presently lacking. The selection lines of house mice have been shown to differ in their expression of the mineralocorticoid and glucocorticoid receptors in the hippocampus (Korte et al.1996, personal communication). In relation to the T dependency of the VP system, differences in

the aromatase activity (enzyme that converts T into estrogen) have been found in these mice as well (Compaan et al. 1993; Compaan et al. 1994a). These two differences may thus be likely candidates to investigate in wild-type rats. The neuropeptide galanin may be considered to be studied since it is known to be colocalized with VP and dependent on T (Miller et al. 1993; Planas et al. 1994a; Planas et al. 1994b; Planas et al. 1994c; Planas et al. 1995; Selvais et al. 1995). Further suggestions for future research can be deducted from results on selection lines of rats in which coping styles have been established as well (see General Introduction, paragraph 3). A disadvantage of the use of selection lines is that it ignores "intermediate" animals, although they really exist. However, by using an unselected population it is possible to investigate *all* individuals, not just the extremes. Finally, it would be tempting to include females into the characterization of coping styles as well.

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